

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:

CHENG et al.

CASE NO: CL2027 US NA

SERIAL NO: 10/735,442

GROUP ART UNIT: 1652

FILED: DECEMBER 12, 2003

EXAMINER: C. FRONDA

FOR: INCREASING CAROTENOID  
PRODUCTION IN BACTERIA VIA  
CHROMOSOMAL INTEGRATION

**AMENDMENT AND RESPONSE TO OCTOBER 16, 2006,  
FINAL OFFICE ACTION**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

This is in response to the Final Office Action mailed on October 16, 2006. A Petition for an Extension of Time under 37 C.F.R. § 1.136(a) and the requisite fee for a two-month extension of time accompany this response. Please amend the above-referenced application as follows and consider the following remarks.

**Amendments to the Claims** are reflected in the listing of claims that begin on page 2 of this paper.

**Remarks/Arguments** begin on page 7 of this paper.

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claim 1 (currently amended): An isolated carotenoid overproducing *E. coli* comprising the genes encoding a functional carotenoid enzymatic biosynthetic pathway consisting of the genes *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB*, *crtE*, *crtB*, *crtI*, and *crtY*; wherein the *dxs*, *idi* and *ygbBP* genes are overexpressed; ~~and wherein the *yjeR* gene is down regulated by gene disruption to have the~~ nucleotide sequence set forth in SEQ ID NO:63; and wherein the *dxs*, the *idi*, *ychB*, *ispA*, *ispB* and *ygbBP* and *yjeR* genes are derived from either *E. coli* or *Methylobacter*; the *dxs* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:13; the *dxr* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:17; the *lytB* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:15; the *crtE* gene is the nucleotide sequence set forth in SEQ ID NO:1; the *crtB* gene is the nucleotide sequence set forth in SEQ ID NO:9; the *crtI* gene is the nucleotide sequence set forth in SEQ ID NO:7; and the *crtY* gene is the nucleotide sequence set forth in SEQ ID NO:5.

Claim 2 (currently amended): An isolated carotenoid overproducing *E. coli* comprising genes encoding a functional carotenoid enzymatic biosynthetic pathway consisting of the genes *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB*, *crtE*, *crtB*, *crtI*, and *crtY*; wherein the *dxs*, *idi*, *ygbBP* and *ispB* genes are overexpressed; ~~and~~ wherein the *dxs*, the *ychB*, *ispA*, *idi*, *ygbBP* and *ispB* genes are derived from either *E. coli* or *Methylobacter*; the *dxs* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:13; the *dxr* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:17; the *lytB* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:15; the *crtE* gene is the nucleotide sequence set forth in SEQ ID NO:1; the *crtB* gene is the nucleotide sequence set forth in SEQ ID NO:9; the *crtI* gene is the nucleotide sequence set forth in SEQ ID NO:7; and the *crtY* gene is the nucleotide sequence set forth in SEQ ID NO:5.

Claim 3 (currently amended): The carotenoid overproducing *E. coli* of Claim 1 or 2 wherein the *lytB* and *dxr* genes are ~~gene is~~ optionally overexpressed ~~and wherein the *lytB* and *dxr* genes are derived from *E. coli* and *Methylobionas*.~~

Claims 4-8 (canceled)

Claim 9 (currently amended): The carotenoid overproducing *E. coli* of either of Claims 1 or 2 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are under the control of a strong promoter.

Claim 10 (previously presented): The carotenoid overproducing *E. coli* of Claim 9 wherein the strong promoter is selected from the group consisting of *lac*, *ara*, *tet*, *trp*,  $\lambda P_L$ ,  $\lambda P_R$ , *T7*, *tac*,  $P_{T5}$ , and *trc*.

Claim 11 (previously presented): The carotenoid overproducing *E. coli* of either of Claims 1 or 2 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are integrated in multicopy in the bacterial chromosome.

Claim 12 (previously presented): The carotenoid overproducing *E. coli* of either of Claims 1 or 2 wherein the *dxs*, *idi*, *ispB* and *ygbBP* genes are present in multicopy in the bacteria on one or more plasmids.

Claims 13-14 (canceled)

Claim 15 (currently amended): The carotenoid overproducing *E. coli* of either of Claims 1 or 2 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are chromosomally integrated into the host cell genome.

Claim 16 (previously presented): A carotenoid overproducing *E. coli* selected from the group consisting of: a strain having the ATCC identification number PTA-4807 and a strain having the ATCC identification number PTA-4823.

Claim 17 (withdrawn): A method for the production of a carotenoid comprising:

- a) growing the carotenoid overproducing bacteria of any of Claims 1-5, the bacteria overexpressing at least one gene selected from the group consisting of *dxs*, *idi ygbBP*, *ispB*, *lytB*, *dxr*, wherein *yjeR* is optionally downregulated, for a time sufficient to produce a carotenoid; and
- b) optionally recovering the carotenoid from the carotenoid overproducing bacteria of step (a).

Claim 18 (withdrawn): A method according to Claim 17 wherein the carotenoid is selected from the group consisting of antheraxanthin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin,  $\beta$ -cryptoxanthin, didehydrolycopene, didehydrolycopene,  $\beta$ -carotene,  $\zeta$ -carotene,  $\delta$ -carotene,  $\gamma$ -carotene, keto- $\gamma$ -carotene,  $\psi$ -carotene,  $\epsilon$ -carotene,  $\beta,\psi$ -carotene, torulene, echinenone, gamma-carotene, zeta-carotene, alpha-cryptoxanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene,  $\beta$ -isorenieratene lactucaxanthin, lutein, lycopene, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, uriolide, uriolide acetate, violaxanthin, zeaxanthin- $\beta$ -diglucoside, zeaxanthin, and C30-carotenoids.

Claim 19 (withdrawn): A method according to Claim 18 wherein the carotenoid is produced at a level of at least about 6 mg per gram dry cell weight.

Claim 20 (withdrawn): A method according to Claim 18 wherein the bacteria is selected from the group consisting of *Agrobacterium*, *Erythrobacter*, *Chlorobium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Deinococcus*, *Paracoccus*, *Escherichia*, *Bacillus*, *Myxococcus*, *Salmonella*, *Yersinia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, and *Myxococcus*.

Claim 21 (withdrawn): A method according to Claim 17 wherein the *dxs*, *idi*, *ygbBP*, *ispB* and *lytB* genes are under the control of a promoter selected from the group consisting of *lac*, *ara*, *tet*, *trp*,  $\lambda P_L$ ,  $\lambda P_R$ , *T7*,  $P_{T5}$ , and *trc*.

Claim 23 (withdrawn): A method according to Claim 17 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are integrated in multicopy in the bacterial chromosome.

Claim 24 (withdrawn): A method according to Claim 17 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are in multicopy in the bacteria on one or more plasmids.

Claim 25 (withdrawn): A method according to Claim 17 wherein the *yjeR* gene is down regulated by gene disruption.

Claim 26 (withdrawn): A method according to Claim 25 wherein the disrupted *yjeR* gene has the nucleotide sequence as set forth in SEQ ID NO:63.

Claim 27 (withdrawn): A method according to Claim 17 wherein the *dxs*, *idi*, *ispB*, *ygbBP* and *lytB* genes are chromosomally integrated into the host cell genome.

Claim 28 (new): An isolated carotenoid overproducing *E. coli* comprising the genes encoding a functional carotenoid enzymatic biosynthetic pathway consisting of the genes *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB*, *crtE*, *crtB*, *crtI*, *crtY*, and *crtZ*; wherein the *dxs*, *idi* and *ygbBP* genes are overexpressed; the *yjeR* gene is down regulated by gene disruption to have the nucleotide sequence set forth in SEQ ID NO:63; the *idi*, *ychB*, *ispA*, *ispB* and *ygbBP* genes are derived from *E. coli*; the *dxs* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:13; the *dxr* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:17; the *lytB* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:15; the *crtE* gene is the nucleotide sequence set forth in SEQ ID NO:1; the *crtB* gene is the nucleotide sequence set forth in SEQ ID NO:9; the *crtI* gene is the nucleotide sequence set forth in SEQ ID NO:7; the *crtY* gene is the nucleotide sequence set forth in SEQ ID NO:5; and the *crtZ* gene is the nucleotide sequence set forth in SEQ ID NO:11.

Claim 29 (new): An isolated carotenoid overproducing *E. coli* comprising genes encoding a functional carotenoid enzymatic biosynthetic pathway consisting of the genes *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB*, *crtE*, *crtB*, *crtI*, *crtY*, and *crtZ*; wherein the *dxs*, *idi*, *ygbBP* and *ispB* genes are overexpressed; the *ychB*, *ispA*, *idi*, *ygbBP* and *ispB* genes are derived from *E. coli*; the *dxs* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:13; the *dxr* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:17; the *lytB* gene is derived from *E. coli* or is the nucleotide sequence set forth in SEQ ID NO:15; the *crtE* gene is the nucleotide sequence set forth in SEQ ID NO:1; the *crtB* gene is the nucleotide sequence set forth in SEQ ID NO:9; the *crtI* gene is the nucleotide sequence set forth in SEQ ID NO:7; the *crtY* gene is the nucleotide sequence set forth in SEQ ID NO:5; and the *crtZ* gene is the nucleotide sequence set forth in SEQ ID NO:11.

## REMARKS/ARGUMENTS

### *Status of the Application*

In the October 16, 2006, Final Office Action, claims 1-5 and 9-15 were rejected. Claim 16 was allowed. In the present response, claims 3, 9, and 15 were amended to correct grammatical errors and for clarity. Claims 1-2 were amended to clarify that the genes encompassed by the claims are either derived from *E. coli* or are as set forth in nucleotide sequences found in the Sequence Listing (support can be found throughout the specification; particularly in the sequence listing; in originally filed claims 4 and 13-14; at page 17, line 29 – page 29, line 13; at page 39, line 28 – page 43, line 28; and in the working examples). Claims 4-5 and 13-14 were canceled without prejudice.

New claims 28 and 29 correspond to canceled claim 5 made independent and directed to the combination of canceled claim 5 with claim 1 (claim 28) and the combination of canceled claim 5 with claim 2 (claim 29). Claim 5 was made independent because the term “consisting of” in the limitation of “consisting of the genes *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *lytB*, *idi*, *ispA*, *ispB*, *crtE*, *crtB*, *crtI*, and *crtY*” would not have allowed the addition of the optional *crtZ* gene to the carotenoid pathway as part of a dependent claim.

Thus, claims 1-3, 9-12, 15-16, and 28-29 are pending. No new matter was added.

### ***Rejections Under 35 U.S.C. § 112, 1<sup>st</sup> Paragraph***

Claims 1-5 and 9-15 were rejected under 35 U.S.C. § 112, 1<sup>st</sup> Paragraph, as failing to comply with the written description requirement. Applicants respectfully submit that the present amendments to the claims, made in accordance with the Examiner’s suggestions from the October 16, 2006, Final Office Action, obviate these rejections.

Claims 1-5 and 9-15 were rejected under 35 U.S.C. § 112, 1<sup>st</sup> Paragraph, because the specification, while being enabling for an isolated *E. coli* host cell transformed with a polynucleotide comprising SEQ ID NO:63 which encodes a mutant oligoribonuclease, where said isolated *E. coli* cell comprises a functional carotenoid enzymatic biosynthetic pathway consisting of proteins of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, and 18; does not reasonably provide enablement for any other

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embodiment as recited in the claims. Applicants respectfully submit that the present amendments to the claims, made in accordance with the Examiner's suggestions from the October 16, 2006, Final Office Action, obviate these rejections.

***Summary***

In view of the foregoing amendments and remarks, Applicants submit that this application is in condition for allowance. In order to expedite disposition of this case, the Examiner is invited to contact Applicants' representative at the telephone number below to resolve any remaining issues. Should there be a fee due which is not accounted for, please charge such fee to Deposit Account No. 04-1928 (E.I. du Pont de Nemours and Company).

Respectfully submitted,

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Date: FEBRUARY 22, 2007